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Pathogenesis of *Pseudomonas aeruginosa* Infection in Chicken

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Abstract

The poultry sector faces a significant threat from *P. aeruginosa* which is considered as an opportunistic microorganism and could be distinguished by its high potential for developing multidrug resistance traits and its capability to secrete multiple virulence factors. Infection with *P. aeruginosa* causes significant financial losses to poultry sector and mainly impacts young birds. Antibiotic resistant strains are predominant that needs treatment with novel or last resort antibiotics as colistin. *P. aeruginosa* is one of the main foodborne pathogens that pose serious risks to food safety because it can contaminate food products causing food poisoning in human. *P. aeruginosa* retains both cell-mediated and secreted virulence determinants. In this review we tried to articulate definition, transmission, pathogenesis, diagnosis, antibiotic resistance mechanisms and control of pseudomonas. Effective control of *Pseudomonas* infection in poultry farms is attained by adopting all necessary biosecurity measures and provide effective antibiotic treatment.

Introduction

Pseudomonas is ubiquitous microorganism frequently exist in soil, water and moist environment; it was also recovered from many origins as (poultry, domestic animals, human and food products) **Handley *et al.*, (2018)**. *Pseudomonas* includes many species whereas *P. aeruginosa* is the most species implicated in poultry diseases; it is an opportunistic pathogen capable to invade different tissues **Shahat *et al.*, (2019)**. It can infect poultry at any age but young ages has higher susceptibility leading to septicemia, and induce high mortalities in baby chicks **Eraky *et al.*, (2020)**. The problem doesn't stand at this limit but it represent a public health hazard as this pathogen is one of the most important zoonotic disease in which chicken carcass and its retail products acts as

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the main source of *P. aeruginosa* transmitted to human **Abd El-Ghany (2021).**

P. aeruginosa is a serious pathogen affecting most avian species representing a bacterial hazard where contaminated hatcheries acts as infection source and infection by this pathogen can induce respiratory manifestation, enteritis, septicemia, keratitis, omphalitis, embryonic death and high mortality rates **Algammal *et al.,* (2023)**.

Since antibiotic residues can contaminate consumed meat, the poultry industry is becoming increasingly concerned about antibiotic replacements due to elevation in antibiotic resistant bacteria and its related public health fears **Abd El-Hack *et al.* (2022).** To ensure the safety of chicken products, a variety of preharvest and postharvest techniques have been developed. Farm-level management practices, such as the feed additives and biosecurity controls. Slaughter and meat processing activities that apply HACCP strategies are considered post-harvest interventions **Tajkarimi (2007).** Presence of many antibiotic resistance mechanisms represents great struggle in *P. aeruginosa* treatment (**Lister *et al*., 2009**).

Pathogen:
P. aeruginosa is Gram negative bacterium, non -sporulated, uncapsulated, and motile by polar flagella, strict aerobe. Microscopically it is a rod shaped bacilli found singly or as short chains, margins of the colonies have hook like projections **Elsayed *et al.,* (2016)**. It usually secrets water soluble green pigment composed of fluorescent and pyocyanin with a characteristics fruity odor**, Urganci *et al.* (2022)**.

Diagnosis:
Isolation:
P. aeruginosa is strict aerobe microorganism that can be cultured on different bacteriological media and produce a grapelike or rotten potato odor. *P. aeruginosa* colonies are spherical, smooth with green color and produce pyocyanin (nonfluorescent blue color) **Eman *et al.*, (2017)**. Following collection of sample it is cultivated on cetrimide, pseudomonas and MacConkey agars and incubated in aerobic conditions. Yellowish-green colonies luminous pigment synthesis is frequently linked to pseudomonads. (**Lamont and Martin, 2003**). Gram' stain was used for morphological identification of the isolates, and many biochemical assays such as (urease, catalase, oxidase, indole, MR, VP, citrate utilization, H2S generation, mannitol fermentation and gelatin hydrolysis) are currently used for biochemical identification **MacFaddin (Williams & Wilkins, 1985)**.

Serotyping:
Serotyping of *P. aeruginosa* isolates showed that detected serotypes are A, B, D, F, H, K, L, and M serotypes. that were recovered from different sources **(El-Gohary *et al.,* 2012)**

PCR identification
Identifying *P. aeruginosa* with traditional means take a lot of time and involve a lot of technician expert hands. Recent molecular techniques have therefore substituted these traditional methods as it proved to be precise, specific, sen-sitive and quick. Polymerase chain reac-tion (PCR) is one of these molecular techniques that can accurately detect specific sequence of *P. aeruginosa* DNA **Eraky *et al.,* (2020).**

Surveillance:
Various investigations have identified *P. aeruginosa* incidence rates in chicken flocks. **Satish and Priti (2015)** isolated *P. aeruginosa* with percent 12% from healthy chicks and 30% from sick ones. **Mohamed (2004**) isolated *P. aurginosa* from broiler chicken flocks in Egypt with percent 17.6%. According to **Saif-Edin (1983)** *P. aeruginosa* was found in chicken in an recovery rate (21.6%**)**. Whereas **Hassan (2013)** iso-lated *P. aeruginosa* from diseased broilers in an incidence 25.3%. Different results were obtained by **Abd El-Tawab (2018)** isolated *P. aeruginosa* in 34% of the inspected broilers. Furthermore low recovery rate estimated by 20% was stated by **Shahat *et al.* (2019)**. Finally it is concluded that *P. aeruginosa* is a serious disease in poultry farms that results in severe respiratory manifestation and high mortalities in broiler **Abd El-Ghany (2021)**. Variation in prevalence rates across

studies may be accredited to the sanitary conditions adopted by each farm, sampling techniques, geographic variation, management practices and factors related to chicken (age and immunity) **Eraky *et al*. (2020).** High sanitary conditions in farms is vital to combat *P. aeruginosa* spread in poultry farms including routine disinfection and cleaning resulting in better outcome as proper infection control. **Abdelmoez *et al.* (2019)**.

Mode of transmission:
Birds are infected by *P. aeruginosa* primarily mechanically via skin damage or by contaminated nee-dles used in vaccination or drug administration. However, course of disease can be affected by several factors as bird's immune status or other associated viral or bacterial infections especially mycoplasma in addition to environmental factors which elevate susceptibility to *P. aeruginosa* infection **Gong *et al*. (2018).**

It is thought that Pseudomonas is the most common organism in processed poultry products. It is supposed that the main source of Pseudomonas infections in humans is retail chicken products. Causes cystic fibrosis and severe lung infections in immune suppressed patients **Handley *et al.,* (2018).**

Pathogenesis:
The pathogenesis of *P. aeruginosa* infections is supposed to occur in 3 stages: (1) colonization, (2) invasion and (3) dissemination and disease induction. This always occurs by interruption of anatomic barriers integrity leading to bacterial invasion, *P. aeruginosa* has some factors that enable it to overcome host immunity, infection by *P. aeruginosa* is acquired through hatchery and is implicated in high mortality rate in newly hatched chicks resulted from yolk sac infections and omphalitis. *P. aeruginosa* preserves cell mediated virulence as(LPS, flagellum, and pilli) all of which play a vital role in motility, bacterial colonization and invasion and help it to reach to their target site strengthening inflammatory response and induce tissue harm **Eraky *et al.* (2020) and Algammal *et al.* (2023).**

A variety of secreted virulent factors for example *pel*A, ***toxA, pslA,*** and ***fliC* genes** is responsible for toxicity and patho-genicity induced by *P. aeruginosa* **Eraky *et al.* (2020)** as thy inhibit protein biosynthesis, aid in biofilm formation, colonization and cell penetration inducing cell death and tissue damage **Ertugrul *et al.,* (2018).**

Virulence factors
P. aeruginosa expressed several virulence factors that promote the establishment and persistence of infection.
1-Biofilm:
Biofilm forming consists mainly of bacterial communities covered by extracellular polymeric substances (EPS) that aid bacteria to attach to any surface **Karatan** and **watnick (2009)**; this phenomena is believed to happen in most bacteria, EPS composed of exopolysaccharides, extracellular DNA (eDNA) and polypeptides that form structural scaffold of biofilm **Chicurel, 2000; Flemming *et al*. (2010) Flemming *et al.* (2007**).

P. aeruginosa is from bacteria forming biofilm under unfavorable conditions, **Shrout *et al.* (2006)**. Life cycle of biofilm in *P. aeruginosa (*PAO1) have 5 major stages. At first, planktonic bacteria adhere to any surface reversibly then adhesion become irreversible then forming microcolonies in EPS matrix which expand and their unions lead to a more structured phenotype with non-colonized space, (stage 3). Non colonized spaces are filled with bacteria and cover the whole surface and the growth of three-dimensional communities is observed in this stage (stage 4). Ultimately bacteria scatter from the sessile structure and revert into planktonic status and retain its activities and maintain its ability to spread and colonize new surfaces **Davey *et al.* (2003)**.

P. aeruginosa produce 3 polysaccharides (Pel, PSL, and alginate) are thought to be the primary factor sustaining biofilm structure **Ghafoor *et al.* (2011)**. Mucoid *P. aeruginosa* is distinguished from non mucoid strains by composition of their polysaccharides in biofilm matrix either they are mainly alginate or Psl/ Pel, respectively **Mann *et al.* (2012)**. According to (**Ertesvag** and **Valla 1998)** alginate is a

linear non branched polymer made up of D-mannuronic acid and L-guluronic acid, providing structural stability and biofilm protection and help retaining of water and nutrients **Sutherland (2001**). The Pel is a matrix that is rich in glucose with vague composition **Franklin *et al.* (2011**). Psl contains pentasaccharide consisting of D-mannose, L-rhamnose, and D-glucose. Pel and Psl acts as the primary construction frame for biofilm matrix **Colvin *et al.* (2011)**. The eDNA play a vital role in early expansion of *P. aeruginosa* biofilms and aids in cell to cell interconnection additionally eDNA constitutes as nutritional source during starvation **Allesen *et al*. (2006**), **Mulcahy *et al*. (2010)**.

Biofilm regulation is highly complicated and comprises multiple bacterial mechanisms (QS systems and 2 regulatory systems) that both interact mainly with EPS production **Kievit *et al*. (2009).** Network defect can lead to in biofilm architecture changes and it can deteriorate its protective role, QS systems (*las* and *rhl*) which control the production throughout synthases LasI and RhlI and the perception by LasR and RhlR**) Rasamiravaka *et al.* (2015)**. The pel gene encodes exopolysaccharide and contains 1–4 linked, partially acetylated galactosamine and glucosamine sugars **Reygaert (2018)**. **The pel** operon regulates the development of a layer of polymer and cells which is known as pellicle formation **Jennings *et al.* (2015)**. The rhlA gene control the next stage of biofilm maturation **Tawakol *et al.* (2018**).

2- Other virulence factors:

Virulence is owed to cellular and extra cellular factors as: (elastase, pyocyanin and pyoverdin production, lipopolysaccharide, alkaline proteases, phospholipase C, haemolysin, biofilm formation, flagellum, and Pilli) in addition to toxin secretion as (S, T, Y, and U exoenzymes). These exotoxins cause continuous effects even after the bacterium is killed by antibiotics **Kirienko *et al.,* (2015).**

Furthermore, *P. aeruginosa* pathogenicity depends on extracellular metabolites including (exotoxins, proteolytic, lipolytic enzymes and pigment) **Bartlett *et al.* (1990)**. Proteases include aeruginolysin, LasA and lasB) that inhibit host inflammatory response to infection **Caballero *et al.* (2001)**.

Cell associated virulence factors together with secreted virulence factors as toxins and protein (ADP-ribosylating enzymes, proteases and phospholipases) gave flexibility to *p. aurginosa* in addition to proteins required for the expression or regulation of molecules in response to stimulus**(Peters and Galloway, 1990)** (**Gambello *et al.,* 1993**).

Virulence related to membranes:

The distal end of LPS a structural component of OM of the majority of Gram-negative bacteria triggers a strong innate inflammatory response and may be capped with O antigen, a long polysaccharide that can range from a few to hundreds of sugars and is essential for bacterial physiology and pathogenesis. LPS can interact directly with hosts and also serve as a target for vaccines. Scientists were initially interested in creating LPS-specific vaccines to prevent infection, but these efforts were ultimately unsuccessful due to the different serotypes and ineffective results **(Maldnoda *et al*. 2016)**. The LPS regulates bacterial interaction with host causing tissue damage and degeneration inducing respiratory infections by airway remodeling **Liu *et al.* (2020)**

Lipoproteins is outer membrane proteins of *P. aeruginosa*, this lipoprotein is restricted to *Pseudomonads* aiding in identification and also acts as an important virulence marker in *Pseudomonas* in clinical specimens **(Remans *et al.* 2010)** and is governed by *opr*L gene which is responsible for intrinsic resistance to antibiotics **(Vanderwoude *et al.* 2020)** in addition to *psl*A and *pel*A genes that play a main role in formation of carbohydrate rich structure of biofilm matrix **(Ghadaksaz *et al.* 2015).**

The pathogenicity of *P. aeruginosa* strains is linked to many virulence factors that are controlled by cell density recognition mechanism called (QS). In addition to Type 3 secretion system (T3SS) and its toxins which are also major virulence determinants of this microorganism known as effectors (ExoU, ExoS, ExoT, ExoY). T3SS is a molecular syringe that

transports virulence effectors directly into the cytoplasm of infected host cells (**Moradali *et al.* 2017).**

According to **Diaz et al., 2011** the transcription of effectors (exoS, exoT, exoU, and exoY) is mediated by 4 regulatory genes which regulate T3SS function, ExoS is 48.3 kDa protein that contributes to cell apoptosis via its GAP region or ADP-ribosyl transferase (**Kroken *et al.* (2022**). Additionally, the ExoS possesses ADPRT activity which induces host cell apoptosis by targeting a variety of Ras proteins. ExoU is the longest *P. aeruginosa* effector composed of 687 amino acids and prompt rapid cell death representing the main driver of cytotoxic phenotype **Hardey *et al.* (2022).** ExoU deregulates innate inflammatory reaction by killing of immune cells (macrophages, neutrophils and others) permitting bacterial persistence, proliferation and spread **(Kaminski *et al.* 2018)**, ExoT has GAP and ADPRT activities and induce host cell apoptosis by targeting Crk proteins phosphorylation that consequently interfere with integrin via destroying focal adhesion sites stability **Wood *et al.* (2015**)

The LuxI/LuxR QS (LasI/LasR and RhlI/ RhLR) genes express many cascade of genes and regulate virulence and biofilm formation **Lee *et al.* (2015**). In a previous study, all *P. aeruginosa* strains possessed QS system genes including those responsible of auto inducers biosynthesis (*lasI* and *rhlI*) **Scaccabarozzi *et al.* (2015**). In a previous study virulence markers in *P. aurginosa* strains of veterinary origin were discussed and by using whole genome analysis assays they grouped *P. aeruginosa* isolates into 3 groups (PAO1, PA14 and PA7), the majority of the tested isolates have been classified to 2 first groups that possess the T3SS machinery *P. aeruginosa* PAO1), *P. aeruginosa* PA14. The third group qualified as clonal outliers included the T3SS-lacking strains that secreted the ExlA **Reboud *et al.* (2017**). *P. aeruginosa* were grouped in three groups according to analysis of 15 different genes that are mainly involved in (virulence, T3SS and QS systems), genes (*lasI*, *lasR*, *rhlI*, and *rhlR*) were amplified in all studied isolates whereas ExoU toxin effector was absent in isolates and other T3SS effectors (ExoS, ExoY, and ExoT) genes were found in 6 isolates **Huber *et al.* (2016)**.

P. aeruginosa produce exotoxin A which is a similar to diphtheria toxin causing leukocytic reduction and liver necrosis **Leidal *et al.* (2003**). Exotoxin A is very toxic for mammalian cells and is able to inhibit protein synthesis of the cell via the ADP-ribosylation of cellular elongation factor 2 leading to tissue damage and facilitate bacterial invasion and is encoded by *toxA* gene **Michalska and Wolf (2015**). Additionally it secrets exotoxin S which is an extracellular protein implicated in cell apoptosis by start of GTPase and ribosyltransferases actions. **Jurado-Martín *et al.* (2021)**.

Based on wealth of available data, it is concluded that *P. aeruginosa* is a multifactorial disease that involves interaction between cell associated factors and secreted virulence determinants **Olejnickova *et al.* (2014**).

Immune mechanisms in response to *P. aeruginosa*:

P. aeruginosa can alter its phenotype and become more skilled at avoiding immune surveillance susceptible to immune mediated killing. *P. aeruginosa* biofilms can modify the immune system in ways that help their survival; for example biofilm protective matrix can act as a physical barrier preventing immune cells from reaching bacterium **Shrout *et al.* (2006)**.

Antibiotic resistance.

The regulatory mechanisms governing the cooperation between virulence factors and antibiotic resistance in pseudomonas are complicated; sometimes these mechanisms are thought to be separate events although from the genetic point of view, such mechanisms are connected and linked **Razzaq *et al.* (2018**), for example *opr*L protein in *P. aurginosa* play a significant role in intrinsic resistance to antibiotics and antiseptics, also biofilm is suggested to be hot spot for accumulation and transmission of AMR genes **Vanderwoude *et al.* (2020**) supporting the hypothesis that there is relation between virulence and antibiotic resistance in *P. aurginosa.*

Great spread of AMR is a worrying emergent affair in human as well as in veterinary field. Egypt has launched a five year National Action Plan on AMR (2017–2022) targeting investigation on AMR and the optimization of antibiotic drug management in human medicine and animal health under the One Health concept adopted by WHO, however until now there is random use for antibiotics in animal feed stuff either as growth promoter or as prophylaxis for infection **Badawy *et al.* (2022).**

Globally spreading multidrug resistance (MDR) bacterial isolates are regarded as public health hazard. XDR and MDR bacterial pathogens from different origins were found to exit **Elbehiry *et al.* (2022).** Antibiotic resistance in *P. aeruginosa* is attributed to intrinsic resistance mechanism due to restricted outer membrane permeability whereas secondary acquired resistance mechanisms as active efflux pump and beta-lactamase giving rise to high level of antibiotic resistance **Hancock and Speert, (2000).**

Acquired resistance in *P. aeruginosa* is attributed to chromosomal mutations or to horizontal gene transfer **Lister *et al.* (2009**). Integrons are mobile genetic elements responsible for resistance gene transfer as they have great ability in capturing, integrating and expressing gene cassettes encoding proteins for antimicrobial resistance. Then these integrons are freely mobile via plasmids or transposons **Haenni *et al.* (2017)**. The antibiotic resistance is variable through low permeability of outer membrane or by acquiring antibiotic resistance genes, and active efflux pumps. In *P. aeruginosa*, the outer membrane proteins (*opr*L) play a significant role in antibiotics and antiseptics resistance **Nikbin *et al.* (2012)**.

Resistance to β-lactam antibiotics is caused by ESBLs that are encoded by ESBL genes, the most prevalent ESBL genes are blaCTX-M and blaTEM, PCR detection of antimicrobial resistance genes is a useful tool **(Menget et al. 2020).** Additionally, the synergism between the blaOXA-1 and blaCTX-M resistance genes supports the resistance to the β-Lactam+β-lactamase inhibitor **Pang *et al.* (2019).**

One of the most important resistance mechanisms was the plasmid-mediated synthesis of enzymes that render antibiotics inactive by hydrolyzing their _beta-lactam rings, which increases the number of point-mutations and resulting in broad spectrum antibiotic resistance **Cooque *et al.* (2008**).

AmpC β-lactamases can be encoded by chromosome or plasmid genes in many gram-negative bacteria that mediate resistance to cephalothin, cefazolin, cefoxitin and β-lactamase inhibitor. Many bacteria have inducible AmpC enzymes that can be mutated to express them at high levels **Jacoby (2009).**

Resistance due to plasmid- mediated AmpC enzymes is less common than ESBL worldwide. AmpC enzymes are evolving to hydrolyze cephalosporins more efficiently, the rapid appearance of β-lactam resistance during drug therapy and frequent therapeutic failures related to expansion of multiple resistances to β-lactam antibiotics are now common **Lambert (2002**).

P. aeruginosa is resistant to aminoglycosides, sulfonamides, and tetracycline due to the occurrence of *aad*A1, *sul*1, and *tet*A resistance genes, respectively **Botelho *et al.* (2019**). In a previous investigation, there is a direct connection between the occurrence of antimicrobial resistance and virulence among the obtained *P. aeruginosa* isolates from broiler **Algammal *et al.* (2023)**. On the other hand, others as **Gajdács *et al*. (2021)** have concluded that there is no correlation between virulence determinants, antimicrobial resistance and biofilm formation in studied *P. aeruginosa* isolates.

Quaternary ammonium compounds (QACs) are active detergents used in poultry industry because of their good antibacterial properties **Minbiole *et al.,* (2016)**. QACs commonly act by distracting the cytoplasmic and outer membrane lipid bilayers and disruption of the critical intermolecular interactions **Tischer *et al.,* (2012)**. Disinfectants have been used randomly leading to adaptation of bacteria to those products and increase bacterial resistance to disinfectants **Loughlin *et al.,* (2002).**

The QACs resistance genes are related to minor multidrug resistance family, *QacED1* is found in combination with genes coding for resistance to sulphonamides, aminoglycosides, chloramphenicol, β-lactams **Zhao *et al.*, (2012)**. It was found that all *qacED1* positive *P. aeruginosa* were resistant to at least three classes of antimicrobial agents, **Bakheet *et al.*, (2017)**. Similarly **Russel (2002)** emphasized that disinfectant resistance is related to antibiotic resistance by coresistance, and co-selection mechanisms.

The occurrence of plasmid mediated efflux pumps could be the factor contributing to fluoroquinolone and β-lactams categories resistance observed in some studies, while tolerance to tetracycline could be correlated to the low permeability of bacterial outer membranes **Tokajian *et al.* (2012)**.

Fluoroquinolones are bactericidal; they are effective against many Gram negative bacteria **Swiatlo *et al.* (2000).** However, fluoroquinolones resistance among *P. aeruginosa* isolates has increased at an alarming manner due to its extensive use, and that can reduce their efficacy **Gasink *et al.* (2006). Kobayashi *et al.* (2013)** found that 6 isolates of *P. aeruginosa* showed DNA fragments of parC and all isolates showing the fragments of gyrA, as the major resistance mechanism to fluoroquinolones in *P. aeruginosa* involving modification of type II topoisomerases (DNA gyrase and topoisomerase IV)

Carbapenems are used for treatment of serious *P. aeruginosa* infections and are regarded as the last-line treatment against MDR) bacterial diseases **Manenzhe *et al.* (2015**). Carbapenem resistance in *P. aeruginosa* is worrying because this class represents the last therapeutic approach. Carbapenems haven't been authorized for use in veterinary field (**OIE 2015**). The exact origins of carbapenemase genes in animals remain vague but this can be due to their mobilization from environmental bacteria into, therefore there is great need to examine carbapenem-resistant *P. aeruginosa* in livestock animals and water and its implication for human health **Elshafiee *et al.* (2019).**

The recent and alarming discovery that some bacteria have acquired resistance to colistin, 'an antibiotic of last resort', confirms that the antimicrobial resistance crisis is now a reality and illustrates the current challenges facing both human and veterinary medicine **Caniaux *et al.* (2017**). This increase in antibiotic resistance is also coupled with decreasing budgets for research and development of new antibiotics **Fernandes (2015).**

Treatment

Colistin is polypeptide antibiotic that is recognized as a critical choice for the treatment of infectious diseases caused by MDR-*P. aeruginosa*. However, the discovery of transferable plasmids conferring colistin resistance genes (*mcr*-1) has created new difficulties in medical science **Caniaux *et al.* (2017).**.

Concerning carbapenems; imipenem has the broadest spectrum action showing activity against both *P. aeruginosa* and all other bacterial species, whereas meropenem exhibits greater efficacy against Gram negative bacteria particularly *P. aeruginosa*. **Manenzhe *et al.* (2015**).

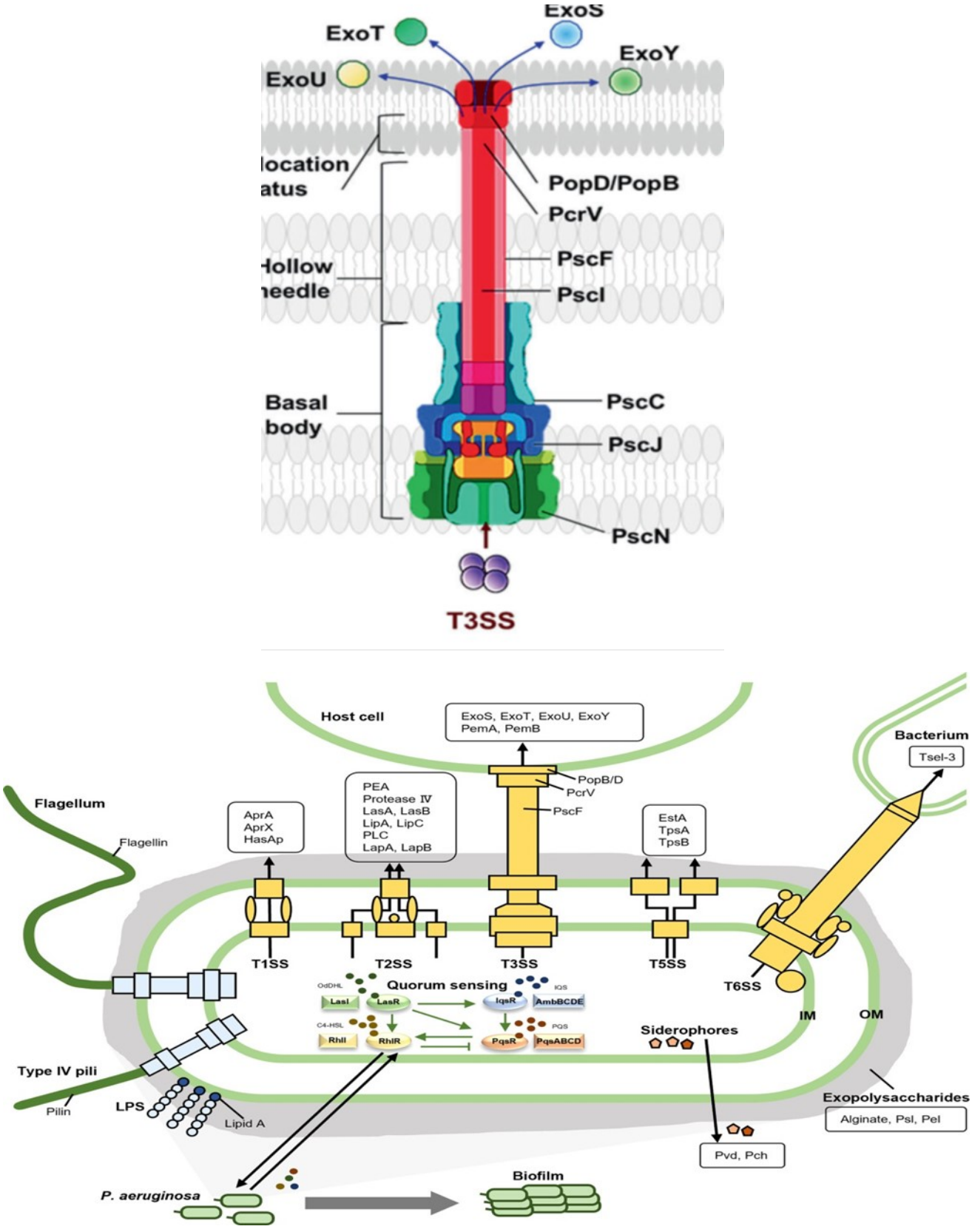

Fig. (1). Virulence of *p. areuginosa* **(Kaminski *et al.* (2018).**

Conclusion and Recommendations

Multidrug-resistant *P.aeruginosa* is a great risk facing poultry industry resulting in high morbidity and mortalities in baby chicks in addition to its zoonotic importance. Consequently there is a great need to control *P.aeruginosa* in poultry flocks by overcoming MDR isolates via effective alternative treatments other than antibiotics side by side with the application of strict hygienic measures in both hatchery and poultry farms. The proper care is needed in the way of careful maintenance and administration of proper antibiotic based on antibiotic sensitivity test. Hence, prevention of *Pseudomonas* invasion is an indispensable duty to any farm.

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